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Pathways of metastasizing intestinal cancer cells revealed: how will fighting metastases at the site of cancer cell arrest affect drug development?



“...therapeutic intervention at time points when cancer cells are already in the circulation or at distant sites are of highest clinical relevance.”

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At present, the dissemination of cancer cells – metastasis – is the primary cause of cancer mortality in more than 90% of cases. Whereas well-confined, primary cancer can be cured by early diagnosis, surgical resection and adjuvant therapy, metastatic disease is incurable in most cases. This is mainly because a metastasizing tumor is a systemic disease affecting various solid organs, the lymphatics as well as the immune system [1]. Thus, a better understanding of the metastatic processes will enable us to identify novel targets, the interference of which may lead to effective treatment of a cancer disease.

Hematogenous metastasis is a complex, sequential process during which cancer cells locally invade the extracellular matrix; intravasate into the blood vessels; survive in the circulation; arrest at distant organ sites; extravasate into the parenchyma of tissues; colonize and remodel the foreign microenvironment and establish metastatic sites [2,3]. It has initially been hypothesized that the accumulation of mutations in various signaling pathways, enabling cancer cells to become invasive, requires a substantial amount of time. However, the fact that metastatic cells have already been detected at early clinical stages of cancer speaks against this hypothesis but does not exclude additional scenarios [2,3].

There is a general consensus that metastatic cancer cells are in a constant ‘cross-talk’ with the local microenvironment, consisting of stromal cells, leukocytes and endothelial cells, and that this ‘communication’ modulates tumor cell migration at primary sites, as well as adhesion

and survival in the circulation and growth of metastatic lesions at distant organs [1,4,5]. To invade the stroma of a primary tumor, carcinoma cells have to breach the basement membrane of epithelial cells that serves as a barrier and has a vital function in the organization of epithelial organs. Furthermore, the extracellular matrix is a reservoir of growth factors and other signaling molecules (e.g., cytokines) whose release by carcinoma-secreted proteases stimulates signaling cascades initiating cell proliferation, survival and invasiveness [6]. Metastasis is determined by dynamic interactions between cancer cells and diverse populations of stromal cells. Besides, bone marrow-derived cells (BMDCs) of myeloid origin are the most prominent cell population present in the tumor microenvironment, at both the primary and metastatic sites [4,7]. Macrophages and BMDCs are capable of modulating the behavior of cancer cells and have been shown to facilitate invasion, intravasation and metastasis in animal models [8–10]. While the contribution of BMDCs to metastasis is becoming more and more accepted, the mode of action and the underlying molecular mechanisms are still under investigation.

From a clinical point of view, in a large number of cancer patients, signs of metastasis (e.g., circulating tumor cells and detectable metastases) are already apparent at the time of diagnosis. Therefore, therapeutic intervention at time points when cancer cells are already in the circulation or at distant sites are of highest clinical relevance.

Keywords

■ CCL2 ■ cell adhesion
■ chemokines ■ colon cancer
■ metastasis ■ tumor
extravasation ■ tumor
microenvironment

Circulating tumor cells must acquire capabilities to survive a variety of stress factors to reach distant organs and to arrest at these sites. Although circulating tumor cells could theoretically disseminate in all organs, clinicians have observed that particular carcinomas metastasize to a limited number of tissues [1]. The nature of this so-called ‘tissue tropism’ of cancer cells remains under investigation, but accumulating evidence suggests that the ‘hosting’ tissue microenvironment critically contributes to organ-specific metastasis [2,11]. Efficient cancer cell adhesion in the vasculature of various organs has been shown to contribute to metastasis efficacy, therefore cell–cell interactions represent useful therapeutic targets for inhibition of this process. Two major cell adhesion molecule families, the selectin and the integrin families, have been described to participate in the metastatic cascade [5]. In addition, a number of soluble mediators (e.g., chemokines), derived from tumor cells, blood constituents and the local microenvironment, facilitate cancer cell extravasation and colonization of distant organs [8,12,13]. Chemokines, a family of small and secreted proteins, play pleiotropic roles in the development and organization of immune responses, as well as in inflammatory-related diseases including cancer [14,15]. In particular, inflammatory chemokines, such as CCL2 and CCL5, are associated with poor prognosis of breast, pancreas and colon cancer due to metastasis, although the mechanisms of action remain under investigation [14–16]. CCL2- and CCL5-mediated recruitment of monocytes has been demonstrated to facilitate metastasis for different cancer types [8,12,17]. Recently, we have demonstrated that CCL2 does not only recruit monocytes to the sites of metastasis but, more importantly, enables cancer cell extravasation [13].

To investigate how CCL2 increases metastasis we analyzed colon cancer metastasis in mice, with or without expression of the chemokine receptor CCR2, which binds CCL2 [13]. First, we analyzed the homing behavior of MC-38GFP colon cancer cells into the lungs of C57BL/6 wild-type (wt) and *Ccr2*^{-/-} mice. Using histological analyses followed by 3D-reconstruction, we showed that MC-38GFP cells did not leave the vasculature in *Ccr2*^{-/-} mice, while in wt mice tumor cells had already extravasated at 24 h post tumor cell challenge. But what could have been the reason for this impaired tumor cell extravasation in *Ccr2*^{-/-} mice? Experiments addressing the status of vascular integrity gave a clue; whereas wt mice displayed a strongly

increased vascular permeability as determined by Evans blue staining, there was no increase in vascular permeability in *Ccr2*^{-/-} mice. Using several techniques and different transgenic mice, we identified two major cell populations expressing CCR2: monocytes and endothelial cells. To define the compartment controlling tumor cell extravasation, we performed reciprocal reconstitution experiments with wt and *Ccr2*^{-/-} mice. We confirmed that CCR2⁺ monocytes/macrophages supported tumor cell metastasis as described previously [8]. However, most unexpectedly, we identified CCR2 expression on endothelial cells as a critical factor for efficient tumor cell extravasation and therefore metastasis.

“In the last decade, prevention of tumor cell metastasis has become one of the most important scientific and clinical aims of cancer research.”

It was not clear whether chemokines activating CCR2 (e.g., CCL2 or CCL7) were produced by host or tumor cells. This question was first analyzed in *Ccl2*^{-/-} mice, which demonstrated lung vascular permeability comparable with wt mice upon tumor cell challenge. Thus, CCL2 produced by host cells was not required for increased vascular permeability, indicating cancer cells to be the source of CCL2. Indeed, MC-38GFP cells, as well as Lewis lung carcinoma cells, expressed high levels of CCL2, which was associated with increased metastasis. Therefore, we hypothesized a link between CCL2 expression on tumor cells and the acquired capacity of tumor cells to successfully extravasate into the lungs. To address this point experimentally, we generated MC-38 and Lewis lung carcinoma cells with a CCL2 knockdown. Indeed, efficient knockdown of CCL2 in both cell lines abrogated their capacity to induce vascular permeability and to efficiently metastasize *in vivo*. Thus, CCL2 expression in cancer cells induces vascular permeability via CCR2 expressed on endothelial cells, which enables efficient tumor cell extravasation.

Finally, we wanted to address the cellular and molecular mechanism(s) of CCL2–CCR2-induced cancer cell extravasation. With the use of an *in vitro* system and various inhibitors we investigated the involvement of signaling cascades in cancer cell extravasation downstream of CCR2. These experiments revealed that, upon CCR2 activation by tumor cell-derived CCL2, JAK2 is phosphorylated, further activating downstream Stat5. In addition, p38

is also activated in order to support efficient tumor cell extravasation. As p38 activation was independent from JAK2 activation, the signaling cascade activating p38 is yet to be defined. Other pathways, such as Stat5, Rac1 and PI3K γ , could be excluded. Our data describe, for the first time, that cancer cell-derived chemokines can activate chemokine receptors (e.g., CCR2) on endothelial cells, and thereby enable efficient tumor cell extravasation. Downstream of CCR2, two independent pathways – in a timely staggered fashion – enable chemokine-driven metastasis. Future experiments will identify the origin of the p38-mediated pathway and the exact cellular kinetics of transmigration.

In the last decade, prevention of tumor cell metastasis has become one of the most important scientific and clinical aims of cancer research. It is therefore not unexpected that accumulating a wealth of data could, at some stage, lead to successful clinical applications. However, efficient inhibition of established or ongoing metastasis by targeting tumor cells is currently a highly complicated and so far unachieved endeavor. Various important points, such as the different behaviors of the distinct tumor types and the pleiotropic/systemic effects of chemokines and other factors (e.g., growth factors and cytokines), have to be addressed first in order to gain a complete understanding of the metastatic process.

“...efficient inhibition of established or ongoing metastasis by targeting tumor cells is currently a highly complicated and so far unachieved endeavor.”

Since cancer metastasis is a cascade of sequential and interlinked events, inhibition of a single step may not be sufficient to completely block metastasis. Furthermore, the time windows to block metastasizing cells from adhering and extravasating into distant organs might be very small, which is supported by experimental

evidence [1,5]. Depending on the stage, cancer patients display a high number of circulating tumor cells and metastasis, therefore a therapy aimed at preventing cancer cell extravasation may strongly reduce the risk of new metastasis, but may not prevent the development of small, already established ones. Nevertheless, accumulating experimental evidence strongly suggests that the interference in cancer cell communication with the microenvironment (e.g., through CCL2) leads to attenuation of metastasis in a number of animal models [8,13,17,18]. Based on this preclinical evidence, two clinical trials have been initiated, evaluating the safety and efficacy of blocking both CCL2 and CCR2 in metastatic patients [101,102]. The initial trial blocking CCL2 proved to be less effective, possibly due to an induced compensatory mechanism that led to an increase of CCL2 expression. The trial targeting CCR2 is currently ongoing. Further clinical trials addressing cancer types associated with high expression of CCL2 and/or CCL5 will test the potential of targeting these chemokines as a potential antimetastatic treatment. Of note, there is currently no antimetastatic therapy clinically available, therefore additional criteria assessing therapy efficacy will be required. Finally, based on the current understanding of the involvement of chemokines in metastasis, cancer patients should be treated from the time of identification of a tumor that is likely to be metastatic, through postoperational treatment.

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